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Update

http://dx.doi.org/10.17545/e-oftalmo.cbo/2017.76

Corneal Cross-Linking: Standard Protocol

Cross-linking da córnea: protocolo padrão

Cross-linking de la córnea: protocolo estándar

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ABSTRACT

The Brazilian Association of Cataract and Refractive Surgery (ABCCR), with this review on the subject, updates the indications of the surgery that promotes new covalent bonds between the fibers of collagen of the cornea, known as Cross-Linking (CXL) and also clarifies their goals.

Keywords:
Cornea;
Keratoconus;
Keratoconus/Prevention & Control;
Keratoconus/Surgical Procedures

RESUMO

A Associação Brasileira de Catarata e Cirurgia Refrativa (ABCCR), com esta revisão sobre o tema, atualiza as indicações da cirurgia que promove novas ligações covalentes entre as fibras de colágeno da córnea, conhecida como Cross-Linking (CXL) e, ainda, esclarece os seus objetivos.

Palavras-Chave:
Córnea;
Ceratocone;
Ceratocone/Prevenção & Controle;
Ceratocone/Procedimentos Cirúrgicos

RESUMEN

La Asociación Brasileña de Catarata y Cirugía Refractiva (ABCCR, por sus siglas en portugués), con esta revisión sobre el tema, actualiza las indicaciones de la cirugía que promueve nuevas conexiones covalentes entre las fibras de colágeno de la córnea, conocida como Cross-Linking (CXL) y, además, aclara sus objetivos.

Palabras Clave:
Córnea;
Queratocono;
Queratocono/Prevención & Control;
Queratocono/ Procedimientos Quirúrgicos

Funding source: None
CEP Approval: Not applicable
Conflicts of interest: None
Received on: December 9, 2016
Approved on: December 12, 2016
Published on: March 31, 2017

How to cite: Santhiago MR. Corneal Cross-Linking: Standard Protocol. e-Oftalmo.CBO: Rev Dig Oftalmol. 2017;3(1):1-10.
http://dx.doi.org/10.17545/e-oftalmo.cbo/2017.76

INTRODUCTION

The finding that keratoconus progression is slower in patients with diabetes and elderly patients indicates that natural cross-linking of corneal collagen fibers could result in the strengthening and hardening of the tissues.[1] This led to the development of corneal collagen cross-linking (CXL), a procedure in which the combination of a photosensitizer, ultraviolet (UV) light and a photochemical reaction leads to the production of free radicals and subsequently to the formation of chemical bonds between the collagen fibers.

A previous study with animals showed an increase of up to 70% in corneal rigidity, [2] whereas the first clinical study conducted by Wollensak et al. showed an interruption of disease progression and corneal flattening in patients with keratoconus.[3] Since then, several prospective studies have found similar results in patients with keratoconus [4,5,6] and corneal ectasia (hereby referred to as "ectasia") after refractive surgery. [7]

Photosensitizer:

Riboflavin acts as an excellent photosensitizer in the biochemical reaction of CXL. Even with a broad range of absorption, it is considered safe for systemic circulation.[8] However, it is a relatively large molecule, and its primary limitation is adequate penetration into the corneal stroma through an intact epithelium, which is essential for its effective action. The standard (or conventional) method that will be discussed in this review consists of the removal of the epithelium. Several CXL techniques with intact epithelium (known as epi-on CXL) have been studied to circumvent this problem, although the efficacy of those protocols is controversial.

UV Light

UV light is the second most important component in CXL. The key parameters include wavelength, fluence, and irradiation time, which are specific for the efficacy and safety of the treatment. Riboflavin absorption peaks for CXL range between 360 and 370 nm, as determined in a previous study.[9] Pre-clinical trials that analyzed variations in intensity and duration have established that maximum rigidity is possible with the use of 3 mW/cm2 of energy for 30 min, which corresponded to a total energy dose (fluence) of 5.4 J/cm2 and resulted in a higher efficacy in terms of tissue thickening. This set of conditions was established as the standard protocol (or Dresden protocol). Variations to this protocol, with higher fluence and shorter duration, were referred to as accelerated CXL.

The photochemical reaction that leads to the actual process of corneal thickening involves the absorption of UV energy by riboflavin and the excitation of the molecule to create an oxygen reactive species. This reaction induces the formation of covalent bonds between corneal collagen molecules and between collagen and proteoglycan molecules,[10] in a process that leads to greater biomechanical rigidity. Oxygen plays a fundamental role in the chemical reaction, although further studies are required to completely understand this relationship and how the duration and the form of exposure to oxygen affect the outcomes of the treatment.

Application of CXL and Indications for the Procedure

Keratoconus

Keratoconus is an ectatic corneal disease that is often bilateral and asymmetric and that mainly results in progressive protrusion and thinning of the cornea. Its onset occurs most often in the second decade of life.[11] CXL has been primarily used to interrupt the progression of keratoconus. Although the protrusion of the corneal stroma is a well-known phenomenon in patients with keratoconus, the underlying pathophysiology of keratoconus is still unknown and appears to be multifactorial.

According to the evidence available in the literature thus far, which will be discussed in detail below, corneal CXL is indicated in patients with progressive disease:

Main Signs of Progression

- An increase of at least 1 D in the keratometry parameters within 12 months.
- An increase of at least 0.75 D in the keratometry parameters within 6 months.
- An increase in myopia of 0.75 D under cycloplegic refraction within 12 months.

- Loss of at least two lines of best-corrected visual acuity within 12 months.

Much has been discussed on how pachymetry or pachymetry mapping can be used to document the progression of the disease and to indicate CXL.

Scientific Evidence of Treatment Efficacy

The first clinical trial on the treatment included patients with progressive keratoconus who underwent the standard (Dresden) protocol, which consisted of administering riboflavin to the de-epithelized cornea before exposure to a source of UV light with an intensity of 3 mW/cm for 30 min, [2,3] The first controlled and randomized studies confirmed the treatment's efficacy in safely interrupting the progression of the disease as well as in promoting flattening, visual acuity improvement, and transient corneal thinning during the first 6 months and improvements in topographic parameters.[8,12] Raiskup et al.[6] published a study with the longest follow-up to date: they monitored patients with keratoconus for 10 years and confirmed corneal stabilization and long-term improvement in visual acuity and topographic parameters. Studies conducted by our group demonstrated that CXL is also effective for patients with advanced stages of keratoconus.[13]

In a recent systematic review and meta-analysis awaiting publication, Santhiago et al. consulted the MEDLINE, CINAHL, Cochrane Library, and EMBASE databases and only analyzed comparative studies. They demonstrated that there is sufficient scientific evidence to state that the standard protocol for CXL is effective in halting disease progression. Fifteen studies compared untreated eyes to eyes subjected to the CXL standard protocol; however, seven publications did not have sufficient data for an adequate analysis.[14,15,16,17,18,19,20] Therefore, our meta-analysis included 396 eyes subjected to CXL, which were evaluated for a period of 12–36 months. These treated eyes were compared to 338 untreated eyes. Other non-comparative studies have corroborated the findings of disease progression interruption and treatment safety. [3,4,5,6,7,12,21,22,23,24,25,26,27]

Given that the onset of keratoconus tends to occur in the second decade of life and that the disease is progressive in nature, pediatric patients may benefit from treatment with CXL.[28] Recent studies have confirmed the efficacy of this treatment as well as significant improvements in topographic parameters and visual acuity.[29,30] However, the analysis of longer follow-up periods suggests that despite the initial improvement, the disease may continue to progress in the long-term in pediatric cases.[31] Additional studies are still necessary and should focus on the development of methods and of an adequate protocol to obtain results that are not only long-lasting and effective but also, and most importantly, safe for pediatric patients. Adequate parental counseling is essential so parents understand the progressive nature of the disease and the need for intervention.

We suggest that CXL be indicated for the following individuals:

- Adult patients with documented keratoconus progression.
- Patients with no diagnosis of ectasia after refractive surgery.
- Patients younger than 18 years of age with no keratoconus diagnosis.

Patients of any age with visual acuity of 20/25 or better may choose to be monitored and not treated because CXL can also lead to complications and reduced vision. In particular, in children who still have adequate vision, the goal and the potential complications of surgical treatment should be discussed and explained before deciding on how to proceed.

Corneal Iatrogenic Ectasia

Corneal ectasia after refractive surgery occurs as a result of biomechanical instability between the collagen fibers and leads to corneal thinning and protrusion similar to keratoconus.[21] It is, by definition, a progressive condition.[32-33]

Initial studies have demonstrated that CXL is effective in interrupting the progression of ectasia [7] and subsequent prospective studies have shown flattening of the corneal topography and improved visual acuity 1 year after treatment.[34] The specific risks of CXL in the treatment of ectasia after LASIK include haze, flap complications, and epithelial growth in the interface.

Assessment of Treatment Efficacy

The primary measure for assessing the results of CXL (i.e., to demonstrate that it interrupts disease progression) is the longitudinal analysis of the corneal curvature. In addition to the keratometry parameters (K), such as maximum K, central K, steepest K, and flattest

K, other variables that indicate disease progression and provide subsequent indications for the treatment should also be used to monitor stabilization outcomes, such as visual acuity and myopia.[12]

Clinically, a stromal demarcation line, which is likely to reflect in the extent of treatment to be indicated, is detected through the use of a slit lamp examination, although it is more easily documented by anterior segment optical coherence tomography and/or confocal microscopy.[35] This demarcation line probably represents the transition between the stroma modified by CXL and the untreated stroma, but it does not confirm the borderline or even the presence of a biomechanical reaction, and it certainly does not necessarily represent the unique changes caused by CXL. The demarcation line does not appear to be associated with changes in visual acuity or maximum keratometry. [36]

Laboratory studies have provided objective data confirming that CXL is in fact effective. These studies have largely relied on electron microscopy visualization of increased diameter of collagen fibers.[37] In addition to higher resistance to enzymatic digestion, there is an increase in biomechanical rigidity indicated by the increase in the Young's modulus [2] which is identified using imaging methods such as atomic-force microscopy [38] and second harmonic generation microscopy. The latter type of microscopy can produce images of the collagen fibrils in a high-contrast mode, thus generating a three-dimensional reconstruction and detecting differences in the patterns of the lamellae between treated and untreated corneas.[39]

The cornea has dynamic elastic properties; therefore, information on its topography only provides a fraction of the data required to determine disease progression, regression, or stabilization. The study of corneal biomechanics provides additional information that improves the determination of CXL efficacy.

Monitoring and Refractive Measurements

We recommend that patients who undergo CXL treatment of corneal ectatic diseases be assessed every 3 months in the first year and every 6 months from the second year onward. In general, measures aiming to correct refractive errors, such as fitting contact lenses after CXL, yield better results after the more intense period of stromal remodeling, which lasts for approximately 12 months. This procedure is suggested by the Brazilian Association of Cataract and Refractive Surgery (ABCCR) and has been adopted by the author of this review.

Safety Parameters and Potential Complications

The safety parameters focus on the protection of ocular structures such as the corneal endothelium, lens, and retina. Firstly, a corneal thickness greater than 400 μm should be considered to protect the corneal endothelium.[40] Once the epithelium has been removed, there is a risk of dehydration, which causes a significant thinning in some cases. Many patients who suffer from corneal ectasia, including keratoconus, already exhibit accentuated thinning before receiving treatment.

Modified protocols use hypoosmolar riboflavin, which promotes fluid retention and causes an increase in thickness of up to approximately 25% of the original value, sometimes reaching the minimum recommended thickness. However, it is unlikely that corneas with values below 350 μm reach a thickness of 400 μm. More recent studies used riboflavin without dextran to allow for the increase in corneal thickness.[41] There is a concern regarding the safety and efficacy of corneas with "iatrogenically modified" thickness because the response to CXL may be lower as a result of the reduced concentration of the collagen fibers in an artificially hydrated cornea.

Another possible explanation for the risk of lower efficacy is the fact that corneal hardening after CXL occurs mainly in the anterior 300 μm; therefore, an excessively hydrated cornea may be too deep for effective treatment.[42]

Additional concerns regarding safety include complications from the treatment itself. The removal of the epithelium creates a variety of risks: corneal infiltrates, late reepithelization, and infectious keratitis. When they occur, corneal infiltrates are sterile and respond to treatment with corticosteroids; they likely represent the body's own antibody reaction to the modification of the corneal tissue.[43]

Although corneal haze following CXL has been studied, its impact on vision has not yet been established. The damage caused to keratocytes is also a concern, even when approaches that preserve the epithelium are used. However, some studies have suggested the occurrence of repopulation within weeks or months after treatment. Other studies have shown that keratocyte apoptosis may be used as an indicator of successful CXL.[44-45]

Remodeling and Flattening after CXL

The remodeling and hardening associated with the healing process following CXL often lead to significant flattening, thinning, and opacity. Many studies, including some conducted by our group,[46,47] have demonstrated that intense flattening may occur following CXL and may even exceed 10 D. The best way to monitor the evolution of this flattening is using differential comparative maps.

The main probable causes of this intense flattening are the localized increase in the tissue elastic modulus, the effective depth of the treatment, and the central location of the cone. [47,48]

The localized increase in the elastic modulus is partly explained by the gradual viscoelastic adjustment to the change in the distribution of tissue stress caused by a selective hardening of the cornea relative to the adjacent sclera. Theoretically, this CXL-associated hardening decreases the central tension of the cornea, which is transferred to the limbus, thus allowing flattening and hypermetropization to occur.

As in other photochemical processes, distinct biological barriers play a role in the efficacy of treatment. In this context, different responses regarding the biomechanical properties or a greater or lesser increase in the elastic modulus are likely explained by the heterogeneity of the disease, which involves an intrinsic interaction between the collagen fibers and the cellular matrix and their orientations. These details are therefore patient-specific. Thus, a greater distance between the collagen fibers and a more defective intertwining allow the photochemical effect of CXL to reach deeper areas and cause a greater increase in corneal rigidity. This is probably the reason why more advanced cases, such as corneas, which are topographically more curved, are more prone to flattening.

The role of the central location of the cone on greater flattening is associated with the anchoring to the sclera at 360o, which allows for a greater distribution of the tensional forces and a greater effect on the curvature.

CXL Protocol with Higher Fluence (Accelerated Method)

According to the Bunsen-Roscoe reciprocity law, the same photochemical effect could be achieved by reducing the illumination time and thus increasing the irradiation intensity. Therefore, 3 min of irradiation at 30 mW/cm2, 5 min of irradiation at 18 mW/cm2, or 10 min of irradiation at 9 mW/cm2 should provide the same effect as that obtained with 30 min of irradiation at 3 mW/cm2. All the combinations of time and intensity result in the same amount of energy (5.4 J/cm2).

There is a substantial variation in the parameters used in the studies that used the accelerated method. The light intensities described vary between 7 and 30 mW/cm2, the irradiation time varies between 3 and 15 min, and the pre-irradiation time of exposure to riboflavin varies between 5 and 30 min. However (and even in the absence of a standard protocol for the accelerated method), all the results demonstrate disease progression interruption; stable keratometric values; and, in some cases, corneal flattening.[49,50,51,52,53,54]

New Indications for CXL

Recent indications for CXL have been assessed in addition to the initial indications aiming to stabilize the ectatic disease process. These include the combination of CXL and excimer laser, referred to as CXL plus[55] and PACK-CXL (photoactivated chromophore for keratitis) using the principles of CXL to treat corneal infections.[56]

CXL Plus

The term CXL Plus refers to the combination of CXL and excimer laser. Topography-guided photorefractive keratectomy (PRK) allows cornea remodeling by theoretically making it more regular without addressing the progressive nature of the disease. Several studies have shown an improvement in visual acuity and stability with the use of topography-guided PRK and CXL. [57]

The efficacy and safety of this type of sequential treatment, the maximum treatment depth, and the use of mitomycin C are still debatable issues. If it is proven to be safe in the long term, the combination of topography-guided PRK and CXL may be a promising method for visual rehabilitation and interruption of disease progression.

In addition to further evaluating the documented potential complications, such as opacity and thinning, there is still much to be studied. Unresolved issues include the adequate combination sequence (i.e., topography-guided PRK first and CXL second or the reverse) and whether the two techniques should be performed in sequence or with an interval between them. Performing topography-guided PRK first and CXL second could contribute to a greater unpredictability of the treatment because of the remodeling process associated with CXL, as has been discussed previously. It would be preferable to perform CXL first, wait for remodeling to occur in

approximately 12 months, and only then perform the topography-guided surgery. In this case, the excimer laser would be used on a theoretically modified cornea (i.e., a hardened cornea), and the ablation rate would be different because hypocorrection would occur. A clearer understanding of the ablation rates of excimer laser after CXL is necessary.

When the excimer laser is used to treat corneas with keratoconus, the most studied and accepted option is topography-guided PRK. Unlike other modalities of conventional PRK or even wavefront-guided PRK, this method consists of a combination of partial myopic PRK in the apex of the cone with hypermetropic PRK in the adjacent area. Therefore, lower amounts of tissue are removed from the thinner and most disease-affected area. Moreover, the main function of the topography-guided treatment is not refractive correction but corneal normalization.

Wavefront-guided treatments appear to be less adequate compared with topography-guided treatments because it is difficult to obtain reliable and reproducible aberrometry examinations in extremely irregular corneas and because the software identifies the most aberrated area (usually the apex of the cone) and attempts to normalize it as much as possible, which may mean excessive thinning in an already affected area.

PACK-CXL

In addition to the use of CXL in the treatment of ectasia, the basic principles of PACK-CXL have been suggested as an option for the treatment of infectious keratitis.[56] The potential benefits include the ability to directly damage the microorganism and increase the resistance to enzymatic damages, prevent microbial replication, release free radicals, and change the surface of the eye with the aim of creating an environment that is hostile to other microorganisms.

In vivo studies on animals have shown the efficacy of PACK-CXL in the treatment of pathogens in complicated keratitis, whereas clinical data have shown keratitis regression with the concomitant use of antimicrobials. [57] The only comparative prospective study that assessed the use of medication alone versus medication associated with PACK-CXL was conducted by Said et al. and showed that the healing time was the same with a tendency toward a more rapid resolution in the PACK-CXL group.[58] A systematic review and meta-analysis of 12 cases studies indicated a shorter healing process in cases of bacterial infection and a worse prognosis and higher risk of transplant in cases of fungi, acanthamoeba, and gram-negative bacteria.[59]

The accelerated protocols also appear to be effective in the elimination of pathogenic agents, thus allowing for a shorter treatment with a similar resolution relative to eliminating pathogens. Some case reports suggest that PACK-CXL alone can aid in the treatment of infectious keratitis, but larger studies are required for confirmation.

Randomized prospective studies comparing treatment with medication versus treatment with medication and PACK-CXL and versus PACK-CXL alone would be ideal, but relevant ethical considerations need to be considered. Nevertheless, PACK-CXL is an option for the treatment of difficult cases of infectious keratitis.

In conclusion, there is sufficient evidence to state that corneal CXL is effective in the stabilization of ectatic disease. The ABCCR has adopted these indications for CXL based on the international literature.

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